

Hybridization, Reticulation, and Species Concepts in the Ferns

DAVID S. BARRINGTON

Department of Botany, University of Vermont, Burlington, Vermont 05405-0086

CHRISTOPHER H. HAUFLER

Department of Botany, University of Kansas, Lawrence, Kansas 66045

CHARLES R. WERTH

Department of Biological Sciences, Texas Tech University, Lubbock, Texas 79409

Hybrids and hybrid species are common among ferns, and they account for many of the problems in species definition in the group. Most systematic inquiry into the evolutionary process in ferns has addressed hybrid species, because meaningful explanations of their origins are feasible (Manton, 1950). As a result, complexes of hybrids, hybrid species, and their progenitor species have been popular subjects for experimental work. Here, we address the definition and changing perception of these hybrid species in the light of improvements in the data available to systematists. Once we have established basic definitions, we demonstrate the utility of recent advances in defining hybrid species of ferns. With this orientation, we investigate the status of hybrid species in the context of reigning species concepts.

Renewed reproductive interaction between populations or species following a period of isolation characterizes all hybrids; hence hybrids are often spoken of as the products of secondary contact. Hybrids are unique in that they arise when isolating mechanisms fail; thus they are evolutionarily a consequence of the disruption of the divergence process that leads to ordinary (primary) species. Consequently, the hybrid is at once a novelty and a rehash: it is a novel combination of genetic and morphological features already present in its progenitors. These features need not be intermediate: see Grant (1975) on transgressive segregation and Barrington, 1986a. Fern hybrids are predominantly sterile (Knobloch, 1976), though there is a small, disparate set of variously fertile hybrids (in *Pteris*, Walker, 1958; in *Dryopteris*, Whittier & Wagner, 1961; in the Cyatheaceae, Conant & Cooper-Driver, 1980). The origin and evolutionary significance of sterile hybrids have been the subject of most studies relevant to a discussion of species concepts in pteridophytes (Lovis, 1977).

Many fern species are thought to be derived from hybrids. Traditionally, these taxa are argued to be 1) species because they breed true and they are autonomous and 2) hybrid because comparison with allied taxa yields evidence of intermediacy. Both allopolyploid and allohomoploid species have been reported in the ferns. The allopolyploid species is an old concept with much experimental support (for a historical synopsis see Manton, 1950). New allopolyploid species ordinarily arise from sterile hybrids via doubling of chromosome sets and consequent restoration of fertility. The typical angiosperm

route to polyploid species, via function of unreduced gametes (deWet, 1980) is by contrast rare in the ferns (but see Gastony, 1986). Allopolyploids have been documented with extensive work in numerous fern genera, especially in the Dryopteridaceae and Aspleniaceae (reviewed by Lovis, 1977). In contrast, the allohomoploid species is an idea new to fern evolutionary biology (Conant & Cooper-Driver, 1980) in need of further testing. New allohomoploid species arise via isolation of fertile F_1 and recombinant hybrids by intragametophytic selfing and/or geographic isolation. Conant and Cooper-Driver (1980) have presented evidence for allohomoploid speciation in species of the Cyatheaceae genus *Alsophila* (including *Nephelea*). The uniform haploid chromosome number of $n = 69$ (Löve et al., 1977) among all scale-bearing Cyatheaceae is consistent with the idea that allohomoploid hybrid speciation has predominated in the family.

To emphasize the significantly different phylogenetic origin of hybrid species, Wagner (1969, 1983) has suggested that hybrid species (nothospecies) should be recognized as qualitatively different from divergent species (orthospecies). He argues that nothospecies are qualitatively different because 1) their origin is a consequence of a return to sympatry and the breakdown of isolating mechanisms, not the action of natural selection and drift on allopatric populations, 2) they originate during one or more discrete episodes each involving only two individual parents, and 3) they are not novelties but combinations of previously existing entities. Wagner's criteria for distinguishing hybrid species are that they have a pattern of character states intermediate between their progenitors, that they be distinct enough from their parents to be treated at the same taxonomic level as their progenitors, and that their reticulate origin be documented. Certainly the origin of hybrid species is distinctive, but distinguishing hybrid species from divergent (primary) species depends on our capacity to recognize the features of hybrid species that document the process by which they originated from their progenitors. Our purpose is to explore the status of taxa now regarded as hybrid species: how have these taxa been treated taxonomically, and do they meet criteria for species definition?

THE RESOLUTION OF HYBRIDS, HYBRID SPECIES, AND RETICULATE COMPLEXES

Successive technical and theoretical advances have led to increased resolution of complexes of hybrids, hybrid species, and their progenitor species. The earliest realization that plants hybridized was based on a morphological criterion—intermediacy (see Wagner, 1983, and Barrington, 1986a, for discussion of intermediacy). For instance, Berkeley (1866) proposed that *Asplenium ebenoides* was *A. platyneuron* \times *A. rhizophyllum*, citing its intermediacy as evidence. Eaton (1879) detailed the characters and invited a test by reconstitution in culture, which Slosson (1902) accomplished in a careful set of experiments. Morphological comparison of reconstituted hybrids with their putative wild counterparts has since been a popular test, especially with European workers (e.g., Walker, 1961; Lovis, 1968). However, morphology alone has not been adequate to allow discrimination of hybrids and hybrid species in complexes with little structural divergence between progenitor species (Paris et

al., 1989). Further, purely morphological criteria do not provide sufficient basis for robust hypotheses about the origin and evolution of hybrid species (Thorpe, 1984).

Morphological criteria related to spore abortion have been used to document fern hybrids (e.g., Tryon, 1948; Wagner & Chen, 1965; Wagner et al., 1986). Features such as collapsed, unopened sporangia, irregular spore shape and size, and failure of indusium eversion have proven to be powerful evidence, in combination with morphological intermediacy, of hybridity.

Evidence from chromosome number and pairing behavior have long provided criteria for recognizing hybrids and hybrid species and distinguishing them from their progenitor species (Manton, 1950). The combination of morphology and chromosomal studies has yielded fully resolved evolutionary hypotheses for several complexes, for example the European polystichums (Manton, 1950) and the Appalachian aspleniums (Wagner, 1954). In these complexes, the diploid progenitor species are all extant and distinctive, and their derived tetraploids are readily discernible using qualitative morphological comparisons.

Summation of biochemical markers provides a powerful basis for discriminating populations of hybrids and hybrid species from phylogenetically patristic intermediates, which can resemble hybrid taxa (Endler, 1977). Two general kinds of markers have been explored, phenolic compounds and isozymes. Interpretation of phenolic data is relatively easy, compared to morphology, because hybrids and hybrid species sum marker compounds characteristic of their progenitor species. For example, Smith and Levin (1963) used chromatography of flavonoids to confirm Wagner's hypothesis for reticulate evolution in the Appalachian *Asplenium* complex. Chromatographic analysis of phloroglucinols has yielded similar confirmations in the genus *Dryopteris* (reviewed in Ew, 1980). However, work on phenolics has proven inadequate to solve problems in many reticulate complexes. Chromatographic patterns are often not clear, the status of chromatographic spots as homologous character states is uncertain, and there is not enough variability in these secondary compounds to provide sufficient species-specific markers.

In the past seven years, allelic variants of isozymes (allozymes) have been used as genetic markers for the study of relationships among hybrid species of ferns. Data on the electrophoretic mobility of these allozymes have been used to 1) confirm or choose between established hypotheses of reticulate relationships, 2) resolve difficulties in poorly understood complexes, and 3) reveal previously unsuspected complexes.

Several examples of hypotheses confirmed or chosen are now available. Comparison of isozymes among species of the Appalachian *Asplenium* complex (Werth et al., 1985a) has confirmed the relationships proposed by Wagner (1954). More recently isozyme evidence has been used to decide the ancestry of *Dryopteris celsa*, an allopolyploid hypothesized as originating from hybridization of *D. ludoviciana* with either *D. goldiana* (Walker, 1962; Wagner, 1971) or *D. marginalis* (Hickok & Klekowski 1975). Werth (1989) presented isozyme data that strongly support *D. goldiana* as the second progenitor of *D. celsa*: the data showed *D. celsa* to sum marker alleles for *D. ludoviciana* and *D.*

goldiana—electrophoretic markers for *D. marginalis* were missing. Gastony (1986, Gastony & Windham, 1989) was able to distinguish between two alternative hypotheses for the allopolyploid origin of *Asplenium plenum* by using isozyme electrophoresis. Recently, Barrington and Conant (unpubl. data) have used isozyme electrophoresis to confirm the hybrid status of the proposed *Alsophila amintae* × *A. portoricensis*, an important first step in testing Conant and Cooper-Driver's (1980) hypothesis for allohomoploid speciation in tree ferns.

Isozyme studies have also been effective in resolving problems in reticulate complexes, especially in those in which some progenitor species pairs are cryptic species (Paris et al., 1989). Haufler has advanced the resolution of the problematic complex in North American *Cystopteris* using isozyme electrophoresis. He demonstrated that the poorly understood *Cystopteris tenuis* (also known as *Cystopteris fragilis* var. *mackayi*) is an allotetraploid species derived from *C. protrusa* of the central Appalachians and a diploid, which although unknown as yet, is also implicated in the origin of the wide-ranging tetraploid *C. fragilis* (Haufler, 1985). The clear qualitative nature of the marker allozymes also allowed the strong inference that *C. tenuis* and *C. fragilis* share a diploid ancestor, a distinction that had not been tenable using only morphological and cytological data.

Finally, isozyme studies have revealed several hybrids and hybrid species that were previously unresolved at any taxonomic level. A previously unsuspected reticulate complex was recently discovered in Central America. Montane polystichums from Costa Rica include an allotetraploid species, *Polystichum talamancanum*, derived from an endemic diploid, *P. concinnum*, and an unknown second progenitor that shares a genome with the Andean tetraploid *P. orbiculatum* (syn. *P. polyphyllum*). The backcross hybrid is now known (Barrington, 1985a) as well as three other hybrids among central American montane species of *Polystichum* (Barrington 1985b and unpubl. data), suggesting that reticulation in the montane tropics progresses much the same as in temperate areas. Isozyme work has also revealed previously unsuspected reticulate evolution in the genus *Adiantum* (Paris & Windham, 1988; Paris et al., 1989).

Recently, morphological discrimination of hybrids and hybrid species has been improved with quantitative analysis of morphometric characters. We provide three examples. Kott and Britton (1982) quantified a suite of morphological features for two *Polypodium* species (traditionally considered as diploid and tetraploid races of *P. virginianum*) and their backcross hybrid, demonstrating that distinctive features characterize the diploid, triploid, and tetraploid. Multivariate statistical analyses provide even better criteria for distinguishing hybrids and hybrid species, because they allow simultaneous unbiased consideration of numerous characters. In several cases, these techniques allowed the discrimination of previously unrecognized or confusing hybrids and hybrid species. For example, Paris and Windham (1988) have documented morphometric distinctions between typical *Adiantum pedatum*, *A. pedatum* subsp. *calderi*, and their derived allotetraploid using both Principal

Components Analysis and Discriminant Function Analysis. In another case, Barrington (1986a) used Principal Components Analysis to provide a clear definition of the differences between triploid *Polystichum* × *potteri* and its tetraploid progenitor *P. braunii*, with which it has commonly been confused. Critical morphometric work, combined with cytological and isozyme analysis, constitute an excellent array of techniques for the resolution of hybrids and hybrid species in polyploid complexes.

As we have improved our perception of hybrids and hybrid species we have come to recognize more species, based on morphological, cytological, and biochemical criteria. In any given epoch, newly resolved taxa are accepted reluctantly, whereas those resolved in the last generation are supported vehemently. To most workers today, *Dryopteris carthusiana* and *D. campyloptera* seem distinct from *D. intermedia*, but specialists argue about the recognition of more recently resolved taxa, such as *Cystopteris laurentiana* and *C. tennesseensis*. Arguments about the correct taxonomic treatment of *Cystopteris tenuis* and the newly discovered allotetraploid ally of *Adiantum pedatum* have hardly begun. The question of relative divergence between progenitors of polyploid species in different evolutionary alliances, which is dependent on a well-documented sample of complexes, including those just being discerned, has yet to be seriously considered.

HYBRID SPECIES AS SPECIES

Do species taxa whose heritage includes hybridization meet criteria as species? We will address the biological reality and taxonomic utility of hybrid species in the context of each of three species concepts; morphological, biological, and evolutionary.

Morphological species are the species of the field biologist and the herbarium taxonomist interested in discerning the diversity of life and developing conservation strategies for them. Hybrids and their derivatives have been difficult to treat as morphological species for five reasons. First, hybrids and their derivative species are confused with their progenitor species because they tend to be intermediate between them, and they often occur with them in similar habitats. Second, hybrid species may originate more than once, combining different structural variants of their progenitor species in each case. Third, at least allopolyploids tend to vary phenotypically toward their progenitor species in habitats where they occur together, the Vavilov effect (see D. Wagner in Barrington, 1985c). For instance, the allotetraploid *Polystichum talamancanum* varies toward its forest-dwelling progenitor in shade, but toward the alpine tetraploid *P. orbiculatum* (with which it shares a genome) when it grows in the sun (Barrington, 1985a and unpubl. data). Fourth, hybrid species commonly hybridize with their progenitors, yielding backcrossed plants that further obscure the morphological boundaries between species. Fifth, anomalous interactions between species yield cytological versions of the same hybrid: triploid and tetraploid versions of *Polystichum* × *potteri* are difficult to distinguish without morphometric analysis (Barrington, 1986b). In spite of these

confusing factors, morphological criteria, corroborated by chromosomal and enzyme data, allow the taxonomist to distinguish hybrid species.

Mayr (1940, 1963) developed the biological species concept based on reproductive isolation and cohesiveness as a consistent category to be used in modern studies of populations and speciation. More recently, botanists have argued that at least among plants the biological species cannot be a real evolutionary entity because plant species evidence neither cohesiveness nor consistent isolation from other evolutionary units (Mishler & Donoghue, 1982). They suggest that we abandon the requirement that species evidence genetic cohesion and autonomy and demand only that species be monophyletic assemblages of populations. Hybrid species are certainly autonomous entities, since many of them reproduce independently of their progenitors. In terms of their reproductive isolation, hybrid species are good biological species, although the origin of the mechanisms isolating them differs from that of divergent species. Allopolyploid species are reproductively isolated from their progenitors because the backcrosses are sterile and therefore do not constitute a means of gene flow between the two progenitors. (However, recurrent polyploidizations do allow gene flow from diploid to polyploid.) Allohomoploid species may be geographically or reproductively isolated from their progenitors (Conant & Cooper-Driver, 1980). Thus, the sympatry involved in the origin of hybrid species is closely followed by the re-establishment of an effective mechanism isolating the hybrid novelty from its allies. Deciding whether or not hybrid species are cohesive will require more extensive data on fern population biology than are now available.

Probably most hybrid species comprise populations derived from different hybridization events (Haufler & Soltis, 1986). For example, the allotetraploids *Asplenium bradleyi* and *A. pinnatifidum* show fixed heterozygosity for different allele combinations (Werth & Windham, in prep.). The apparent parallel origin of allotetraploids such as *Dryopteris campyloptera* (*D. austriaca*) in Europe and North America (Gibby, 1977) represents an extreme case of multiple origins that presents a challenge to applying the biological species concept to hybrid species. On the one hand, populations on two continents have a negligible chance of interacting reproductively, and the allotetraploid will continue to be generated from the diploids in each place as long as the two can hybridize. On the other hand, a careful analysis of the genetic and structural features of the allotetraploids and their diploid progenitor populations on both continents sometimes reveals that they are genetically and morphologically indistinguishable: Walker (1961) argued on the basis of synthesized hybrids that *D. intermedia* of eastern North America and *D. maderensis* of Madeira are conspecific. Comparative analysis of more allopolyploid populations from different continents is needed to resolve this issue. Note that hybrid species showing multiple origins would fail the modified species concept of Mishler and Donoghue (1982), since they are demonstrably polyphyletic.

Simpson's evolutionary species concept (Simpson, 1961) has been developed for plant biologists by Grant (1981) and applied to the needs of systematists using cladistic methods by Wiley (1981). Hybrid species meet the criteria for

evolutionary species: they have spatio-temporal identity and their own evolutionary tendencies and historical fate. However, they are unusual in that they originate without divergence. Unlike divergent species, hybrid species are derived from two lineages, not one, and they can displace their progenitors over time (Stebbins, 1971). This displacement may be of major importance in the ferns: allopolyploid ferns may be predominantly selfing and thus better at migration than their predominantly outcrossing progenitors (Haufler, 1989). Regardless of their origin and fate, hybrid species are spatio-temporal lineages, and thus are evolutionary species.

Are there two distinguishable kinds of species, hybrid species (nothospecies) and divergent species (orthospecies), as Wagner contends (1969, 1983)? In the case of the allopolyploids, the answer to this question depends on an understanding of chromosomal evolution in the ferns. High chromosome numbers in the ferns have been interpreted in two ways: one school argues that they are the result of repeated polyploid speciation—progenitor extinction events (Haufler, 1987; Werth & Windham, in prep.), the other that they are primitively high (Haufler & Soltis, 1986; Soltis & Soltis, 1987), perhaps because selection for a greater number of linkage groups would reduce problems of homozygosity (Buckley & Lloyd, 1985). Development of the argument for repeated polyploidization depends on distinguishing between neo- and paleopolyploids (Wagner & Wagner, 1980). Neopolyploids are documented recent allopolyploids with chromosome numbers that are multiples of the lowest number now known for their evolutionary group; paleopolyploids are hypothesized ancient allopolyploids that have the lowest known chromosome numbers for their evolutionary group (that is, the base number)—they include the progenitors of the neopolyploids. Ferns with the lowest chromosome numbers for their genus have so far consistently shown diploid gene expression (Haufler & Soltis, 1986; Haufler, 1987; Wolf et al., 1987). If there have been repeated cycles of polyploidy, then there must have been consistent, exhaustive diploidization (gene silencing) of the paleopolyploids and concomitant extinction of the progenitor diploids. If gene silencing has indeed had a major role in fern evolution, it probably constitutes an isolating mechanism facilitating divergent evolution among allopolyploid species, since two populations, each silenced for reciprocal alleles of the same gene, would yield hybrid sporophytes with reduced gametophyte viability (Werth & Windham, in prep.). Consequently, hybrid speciation (secondary speciation) would be succeeded by speciation at the polyploid level (*tertiary speciation*—Haufler, 1989). Some evidence of silencing has begun to accumulate (Werth et al., 1985a,b; Elisens & Crawford, 1988; Werth & Windham, in prep.), and further inquiry may demonstrate that gene silencing has been important in polyploid evolution. At the moment neither interpretation of high basic chromosome numbers in the ferns is unequivocally supported: both should be further tested.

If there have been repeated cycles of polyploidy in the evolutionary history of ferns, many species dubbed orthospecies (because they have the lowest known chromosome number for the genus) may be diploidized nothospecies. The problem is that all of the criteria for recognizing nothospecies are comparative:

they require analysis of both the hybrid species and its progenitors. Paleopolyploids cannot be identified as hybrid versus divergent species, since they have the base number for their evolutionary group, and their diploid progenitors must be extinct. The nothospecies is useful if neopolyploids are a new kind of species distinct from their progenitors. However, it is not useful if paleopolyploidy has had a role in the evolutionary history of fern species, since detectable nothospecies (neopolyploids) would simply be the latest products of an ongoing, uniform process.

CONCLUSIONS

Perception of the taxa arising from hybridization and allopolyploidy has changed dramatically in the last 120 years, and it is likely to continue to change as we develop more tools for discerning interactive, derivative species from their progenitors. From our synthesis, we conclude that hybridization yields species, be they allohomoploid or allopolyploid, since these entities meet morphological and biological criteria. Though the question, "Are hybrid species good species in the evolutionary sense?" requires more thought, the answer appears to be "Yes." Hybrid species are lineages in space and time, in spite of their atypical origin and extinction. Whether there are two qualitatively different kinds of species, nothospecies and orthospecies, depends on whether there have been repeated cycles of polyploidy, since the nothospecies is a qualitatively different and useful concept only if there have not been repeated cycles. If tertiary speciation via reciprocal gene silencing is a significant mode of generating diversity, then hybrid species may be more similar to divergent species in evolutionary potential than we now have evidence to claim. We recommend that hybrid taxa be treated as species if they meet the criteria for species and they can be discerned using the techniques now generally available to systematists.

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